Structural features of the gene encoding human muscle type carnitine palmitoyltransferase I

Naoshi Yamazaki[a], Yasuhisa Yamanaka[a], Yoshiko Hashimoto[a], Yasuo Shinohara[a], Atsushi Shima[b], Hiroshi Terada[a,*]

[a] *Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770, Japan*
[b] *Nutrition Research Institute, Otsuka Pharmaceutical Factory, Inc., Naruto 772, Japan*

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Abstract We isolated a human muscle type of carnitine palmitoyltransferase I (CPTI-M) genomic clone and determined its entire nucleotide sequence. By comparison of the nucleotide sequence of the genomic clone with that of cDNA, we determined the intron/exon junctions. For detection of the exon(s) in the 5′-region of the CPTI-M gene, we isolated cDNA clones corresponding to the 5′-region of its transcript by 5′-rapid amplification of cDNA ends (5′-RACE method). Results showed two alternative exons, 1A and 1B, that do not encode amino acids in the 5′-region of the human CPTI-M gene. The gene encoding human CPTI-M was found to consist of two 5′-non-coding exons, 18 coding exons and one 3′-non-coding exon spanning approximately 10 kbp. Furthermore, on analysis of the 5′-flanking region, a putative gene encoding a 'choline kinase homologue' was found to be located only about 300 bp upstream from exon 1A of the human CPTI-M gene. Comparison of the gene structure of human CPTI-M with the reported partial gene structure of human liver type CPTI (CPTI-L) showed that the intron insertion sites were completely conserved in these two genes.

Key words: Carnitine palmitoyltransferase I; Gene structure; Carnitine system

1. Introduction

For mitochondrial β-oxidation of long-chain fatty acids, fatty acids must be transported into the matrix space of mitochondria. Before entering the mitochondria, they are metabolized to long-chain acyl-CoAs, but they are not able to penetrate into the mitochondrial matrix space across the inner membrane. For their transport, acyl-CoA is converted to acyl-carnitine by carnitine palmitoyltransferase I (CPTI). Acyl-carnitine transported into the mitochondrial matrix space via the carnitine carrier is reconverted to acyl-CoA by carnitine palmitoyltransferase II (CPTII), and functions in β-oxidation (for reviews, see refs. [1–3]). The activity of CPTI is strictly regulated by a substrate for fatty acid synthesis, malonyl-CoA. Therefore, CPTI is regarded as the first rate-limiting enzyme in fatty acid β-oxidation [1–3].

To date, two isoforms of CPTI, liver-type CPTI (CPTI-L) and muscle-type CPTI (CPTI-M) have been identified. CPTI-L is expressed mainly in the liver, kidney and fibroblasts, and to a lesser extent in the heart. CPTI-M is expressed extensively in skeletal muscle, heart and brown adipose tissue. Recently, cDNA clones encoding CPTI-L [4,5] and CPTI-M [6,7] were isolated from rat and human tissues and their nucleotide sequences were determined. However, no genes encoding CPTI isoforms have yet been isolated and characterized except for a part of the human CPTI-L gene [5].

Fatty acids are a major source of bioenergy for heart, skeletal muscle and brown adipose tissue, in which CPTI-M is abundantly expressed. Accordingly, the role of CPTI-M in these tissues is of great importance. However, as the structure of the gene encoding CPTI-M has not been determined, the mechanism of regulation of CPTI-M expression is not yet understood. This paper deals with the structural characteristics of the gene encoding human CPTI-M.

2. Materials and methods

2.1. Materials and general procedures

A genomic DNA library of human placenta and poly(A)$^-$ RNAs of human tissues were obtained from Clontech (Palo Alto). Reverse transcriptase and terminal deoxynucleotidyl transferase were from Gibco BRL (Gaithersburg), and *Taq* DNA polymerase was from Takara Shuzo (Kyoto). All other reagents and enzymes were obtained as described previously [6]. Recombinant experiments were carried out according to the standard method of Sambrook et al. [8].

2.2. Isolation and nucleotide sequencing of genomic DNA of human CPTI-M

The genomic clone HG11 encoding human CPTI-M was isolated from a genomic DNA library of human placenta as reported previously [7]. The inserted DNA of HG11 was digested with restriction endonucleases and subcloned into plasmid vectors. The nucleotide sequence of human CPTI-M was determined by the chain-termination method using [α-^{32}P]dCTP or fluorescent primers. In the latter case, the nucleotide sequence was analyzed in an ALF^{TM} DNA sequencer (Pharmacia).

2.3. Isolation of cDNA clones including the 5′-region by 5′-RACE

The 5′-region of the cDNA of human CPTI-M was isolated by 5′-RACE [9] based on the nucleotide sequence of cDNA encoding CPTI-M [7]. For this, we prepared synthetic primers of GSP1 (5′-TGGTCAAGTTGCTGGTCTTG, position 429–448, antisense), GSP2 (5′-TTGCTGGTCTTGCCATGC, position 423–440, antisense), GSP3 (5′-GAGGATCCTGCATCTCAAACATCCACC, position 407–425, antisense, with addition of a *Bam*HI site at the 5′-terminus), T_{17}Adp (5′-GAGTCGACTCGAGAATTCT$_{17}$) and Adp (5′-GAGTCGACTCGAGAATTC). The positions of these primers are

*Corresponding author. Fax: +81 (886) 33-5195.
e-mail: hterada@ph.tokushima-u.ac.jp

Abbreviations: CPTI, carnitine palmitoyltransferase I; CPTI-M, muscle-type carnitine palmitoyltransferase I; CPTI-L, liver-type carnitine palmitoyltransferase I; CPTII, carnitine palmitoyltransferase II; RT-PCR, reverse transcription followed by the polymerase chain reaction; RACE, rapid amplification of cDNA ends; bp, base pair(s)

The nucleotide sequence data reported in this paper have been submitted to the GSDB, DDBJ, EMBL and NCBI Nucleotide Sequence Databases under accession number AB003286.

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according to Ref. [7]. Poly(A)$^+$ RNAs of human heart and skeletal muscle were reverse-transcribed using GSP1, and poly(dA) was added to the 3′-end of the synthesized first-strand cDNA using terminal deoxynucleotidyl transferase. The first PCR was carried out using this cDNA as a template and GSP2 and T_{17}Adp as primers. The second PCR was carried out using the primers GSP3 and Adp. Various cDNA fragments amplified by PCR were pooled and ligated to a plasmid vector, and the plasmids obtained were transformed into *E. coli*. The positive recombinants detected by Southern analysis with use of a cDNA probe of human CPTI-M were isolated and their nucleotide sequences were determined.

2.4. Northern blotting using 5′-upstream regions of the human CPTI-M gene

The possible existence of an exon(s) in the 5′-upstream region was examined by Northern analysis with genomic DNA fragments obtained by digestion with appropriate endonucleases. Samples of 0.5 μg of human heart and skeletal muscle poly(A)$^+$ RNAs were subjected to denatured agarose gel electrophoresis and transferred to nitrocellulose membranes.

3. Results and discussion

Previously, we isolated the genomic clone HG11 containing the human CPTI-M gene from a genomic library of human placenta [7]. In this study, we first examined whether HG11 contains the entire gene encoding CPTI-M. Southern blot analysis with cDNA probes of several parts of human CPTI-M showed that HG11 seemed to include the entire gene (data not shown). Then, for determination of the structure of the gene encoding human CPTI-M, the inserted DNA of the HG11 was digested with several restriction endonucleases, the DNA fragments obtained were subcloned into plasmid vectors, and the nucleotide sequences of these plasmids were determined. Fig. 1 shows the nucleotide sequence of the human CPTI-M gene and its 5′- and 3′-flanking regions. We found several misinterpreted nucleotides in the nucleotide sequence of human CPT-M cDNA reported by us previously [7], which are shown in bold face in Fig. 1 (for details, see legend of Fig. 1).

Previously, we isolated cDNA clones encoding human CPTI-M from a cDNA library of human heart. However, on comparison of the nucleotide sequence of human CPTI-M with that of rat CPTI-M cDNA [6], we found that the isolated human CPTI-M cDNA clones were devoid of the 5′-region of the open reading frame. Therefore, we determined the nucleotide sequence of the 5′-region of CPTI-M cDNA from the nucleotide sequence of the human genomic clone HG11 taking into consideration the molecular size of human CPTI-M and the amino acid sequence of rat CPTI-M [7].

In this study, we carried out 5′-RACE to determine the exact nucleotide sequence of the 5′-region of the cDNA encoding human CPTI-M. The nucleotide sequence of the cDNA fragments obtained by 5′-RACE was compared with that of the corresponding region of the human CPTI-M gene. The results showed two exons in the 5′-upstream region of the translation initiation codon, which we refer to as 'exon 1A' and 'exon 1B'. In exon 1A, we found an additional 'ATG' both in the genomic DNA and 5′-RACE clone of human CPTI-M. However, according to the Kozak rule [10], the nucleotide sequence surrounding the 'ATG' should be A/GXXATGG for efficient translation initiation in mammals. This consensus sequence was not observed in the region containing the 'ATG' sequence in exon 1A, but the surrounding sequence of the 'ATG' in the 2nd exon, which was regarded as a translation initiation codon [7], conforms to the Kozak rule. Therefore, we concluded that exons 1A and 1B are non-coding exons, and only the 'ATG' in the 2nd exon is used as a translation initiation codon.

We obtained two species of 5′-RACE products containing either exon 1A or exon 1B, but never a product containing both 1A and 1B. Therefore, we concluded that these two non-coding exons are transcribed in an alternative manner. There should be two possibilities for the alternative existence of exons 1A and 1B in 5′-RACE products: (1) the two exons are transcribed by different promoters or (2) they are spliced alternatively after initiation of transcription from an unidentified exon(s) located in a further upstream region. For determination of which mechanism is effective, the transcription initiation site should be determined. However, exact determination of this transcription initiation site is difficult by the 5-RACE.

Therefore, to characterize the 5′-flanking region of this gene, we carried out homology search analysis of the nucleotide sequence of this region using the BLAST program [11]. The results showed that the nucleotide sequences of two cDNA clones registered with accession numbers T07548 and T30127 were the same as those of the corresponding region of the upstream of exon 1A of the human CPTI-M gene. These two human cDNA clones are registered as the cDNA encoding a 'human choline kinase like protein', although no functional characterization of these cDNA clones has been performed. By comparison of the nucleotide sequence of the registered cDNA clones with that of the 5′-upstream region of the human CPTI-M gene, we determined putative intron/exon junctions of the gene encoding this 'human choline kinase like protein' to be as shown in Fig. 1. Interestingly the 3′-end of the gene encoding the 'choline kinase like protein' is located only 314 bases from exon 1A of the CPTI-M gene.

We next carried out primer extension to determine the transcription initiation site. However, we did not obtain the appropriate reverse-transcribed products, possibly because the transcript took a secondary structure. We then examined the possible existence of a further exon(s) in the 5′-flanking region by Northern blotting using various genomic DNA fragments

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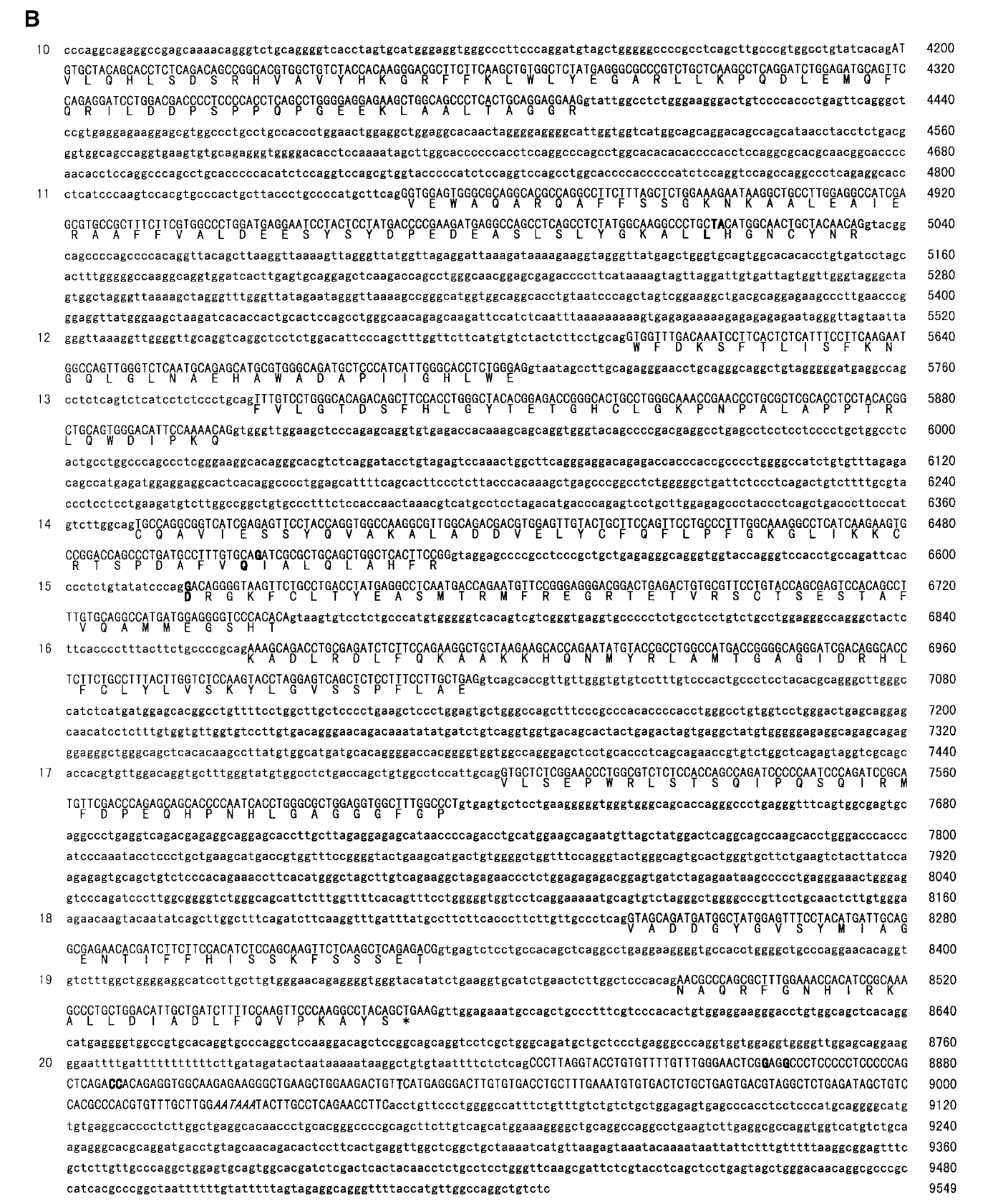
Fig. 1. Nucleotide sequence of the human CPTI-M gene and its flanking regions. The adenine base in the translation start codon of human CPTI-M is numbered as +1 and the numbers of the nucleotides are shown in the right margin. Nucleotides in the exon and intron are shown by upper and lower case letters, respectively. Exons of the CPTI-M gene are numbered in the left margin. The deduced amino acid sequence is shown by the one-letter abbreviation code under the nucleotides in the exon. The sequence 'AATAAA' shown in italics represents a putative poly(A)$^+$ additional signal. The revised nucleotide and amino acid sequences of human CPTI-M, which were misinterpreted in our previous report [7], are shown in bold face. The nucleotides between −54 and −20, shown in bold lower case letters, are those we reported previously [7] as constituents of cDNA. The revised sequence was registered with the accession number D87812. The nucleotides in the exons identified by 5′-RACE are shown in bold upper case letters in the upstream of the translation start codon. The gene encoding a 'choline kinase like protein' was located in the upstream region from −1060, and the nucleotides in its exons and poly(A)$^+$ additional signal are shown in upper case and italic letters, respectively. The structural gene encoding human CPTI-M is from −745 to 9046.

as probes: those containing an upstream region of exon 1A to the 3′-end of the 'choline kinase like protein' (HP160, PP120 and HP280), exon 1A (PS250), exon 1B (SS280), and a downstream region from the 3′-end of the 'choline kinase like protein' (HH1100). As shown in Fig. 2, a hybridization band was observed when the transcript was hybridized with probes containing exon 1A and/or 1B, but not with probes containing upstream regions of exon 1A, showing that there was no exon

A



in the upstream region of exon 1A of the human CPTI-M gene. Therefore, we concluded that the structural gene encoding human CPTI-M starts from the 5′-end of exon 1A (nucleotide −745) and ends at the 3′-end of exon 20 (nucleotide 9046, see Fig. 1).

Recently, a partial structure of the gene encoding human

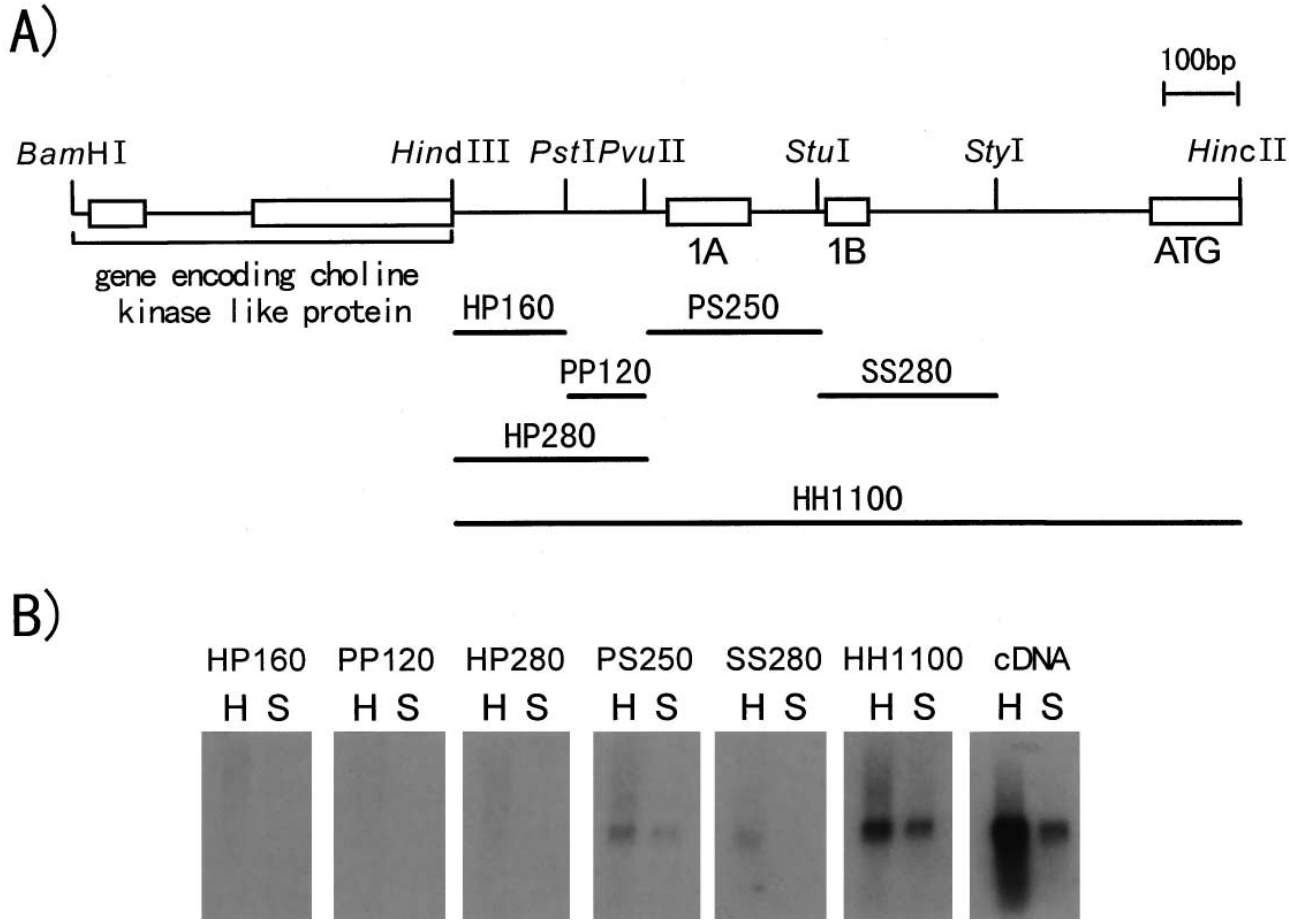


Fig. 2. Northern blot analysis of transcripts using genomic DNA fragments as probes. A: Loci of restriction DNA fragments of the human CPTI-M gene used as probes. The top row indicates the 3′-part of the putative gene encoding a 'choline kinase like protein' and the following 5′-region of the CPTI-M gene. Exons are shown by boxes. The probes were prepared by digestion of inserted DNA of HG11 with *Hin*dIII and *Pst*I (HP160), *Pst*I and *Pvu*II (PP120), *Hin*dIII and *Pvu*II (HP280), *Pvu*II and *Stu*I containing exon 1A (PS250), *Stu*I and *Sty*I containing exon 1B (SS280), and *Hin*dIII and *Hin*cII containing exons 1A, 1B and a part of exon 2 (HH1100). B: Results of Northern blotting. Samples of 0.5 μg of poly(A)$^+$ RNAs of human heart and skeletal muscle were analyzed with the probes shown in (A). H and S show results with RNA samples of heart and skeletal muscle, respectively. 'cDNA' shows the result with a cDNA fragment of human CPTI-M [7] as a positive control.

liver type CPTI (CPTI-L) was reported by Britton et al. [5]. We compared the gene structure of human CPTI-M with that of CPTI-L. As shown in Fig. 3, three intron insertion sites were found to be completely conserved in these CPTI isoforms. However, the size of the intron between the 6th and 7th exons of human CPTI-M (85 bp) was markedly different from that of the corresponding intron of human CPTI-L (2.3 kbp). For a more detailed comparison, the complete gene structure of human CPTI-L is necessary. On recent chromosomal assignment of human CPTI isoforms, these proteins were found to be encoded by genes located in separate positions [12]. Therefore, it is possible to speculate that the ances-

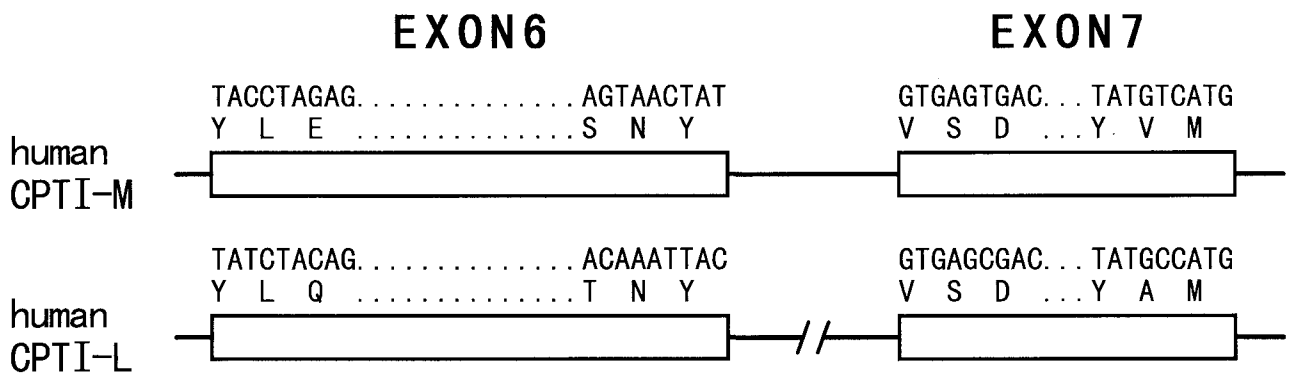


Fig. 3. Comparison of the gene organizations of human CPTI-M and CPTI-L. Exons of the human CPTI-M and CPTI-L genes are shown by boxes, and the nucleotides located in the edges of exons and the deduced amino acids are shown according to Refs. [7] and [5], respectively. Intron insertion sites and sizes of exons are completely conserved, at least in the regions analyzed.

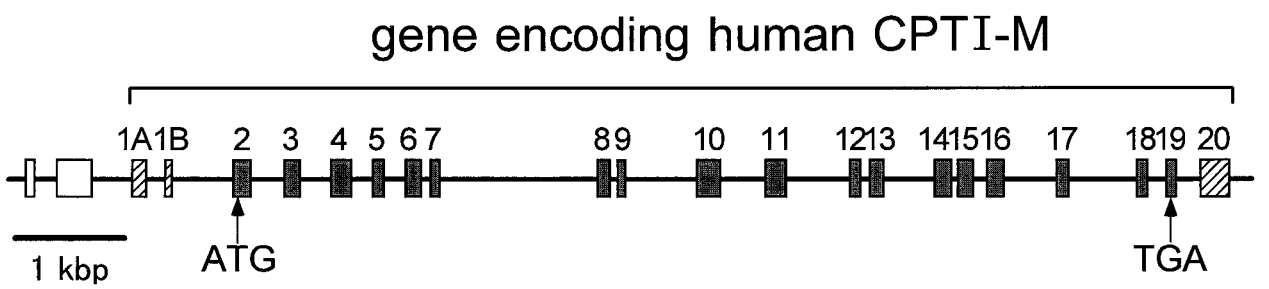


Fig. 4. Structural features of the human CPTI-M gene and its flanking regions. Non-coding and coding exons of the human CPTI-M gene are shown by hatched and closed boxes, respectively, and exons are numbered above the boxes. Two exons of the gene encoding a 'choline kinase like protein' observed in the flanking region of the human CPTI-M gene are shown by open boxes.

tral gene of CPTI isoforms already containing introns was duplicated and inserted into different positions of the chromosome, and that during development the detailed structures of the isoforms were altered to show different enzymatic activities and tissue distributions.

In this study we determined the structural features of the gene encoding human CPTI-M. We found that the structural gene of human CPTI-M started from the 5′-end of exon 1A, and that its transcription was initiated from either exon 1A or exon 1B. Therefore, it consisted of two alternative 5′-non-coding exons, 18 coding exons and a 3′-non-coding exon spanning about 10 kbp, as shown schematically in Fig. 4. In addition, a gene encoding a 'choline kinase like protein' was found to be located only about 300 bp upstream from the 5′-end of exon 1A of the CPTI-M gene. As far as we know, such a close location of two different genes is very rare in the mammalian genome. The results of Northern blot analysis (Fig. 2) showed that both exons 1A and 1B are used for the transcription of CPTI-M in heart and skeletal muscle. This was confirmed by exon 1A and 1B specific RT-PCR (data not shown). Although tissue-dependent alternative use of these exons was not observed in the heart and skeletal muscle, it is possible that either of the promoters of these exons responds effectively to changes in physiological conditions. For a full understanding of the transcription mechanism, an exact determination of the transcription initiation site(s) and measurements of promoter activity are very important, and these studies are underway.

References

[1] McGarry, J.D., Woeltje, K.F., Kuwajima, M., Foster, D.W., Diabetes/Metab. Rev. 5 (1989) 271–284.
[2] McGarry, J.D., Biochem. Soc. Trans. 23 (1995) 321–324.
[3] McGarry, J.D., Brown, N.F., Eur. J. Biochem. 244 (1997) 1–14.
[4] Esser, V., Britton, C.H., Weis, B.C., Foster, D.W., McGarry, J.D., J. Biol. Chem. 268 (1993) 5817–5822.
[5] Britton, C.H., Schultz, R.A., Zhang, B., Esser, V., Foster, D.W., McGarry, J.D., Proc. Natl. Acad. Sci. USA 92 (1995) 1984–1988.
[6] Yamazaki, N., Shinohara, Y., Shima, A., Terada, H., FEBS Lett. 363 (1995) 41–45.
[7] Yamazaki, N., Shinohara, Y., Shima, A., Yamanaka, Y., Terada, H., Biochim. Biophys. Acta 1307 (1996) 157–161.
[8] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[9] McPherson, M.J., Hames, B.D. and Taylor, G.R. (1995) PCR2: A Practical Approach, IRL Press, Oxford.
[10] Kozak, M., Nucleic Acids Res. 9 (1981) 5233–5252.
[11] Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., J. Mol. Biol. 215 (1990) 403–410.
[12] Britton, C.H., Mackey, D.W., Esser, V., Foster, D.W., Burns, D.K., Yarnall, D.P., Froguel, P., McGarry, J.D., Genomics 40 (1997) 209–211.